VIRULENT MICROCOCCUS CATARRHALIS IN INFLUENZA

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Several points of bacteriologic interest were brought out during the 1920 epidemic of influenza at the University of Wisconsin. During this epidemic, bacteriologic examination was made of the nasopharyngeal flora of 79 patients suffering from influenza, 15 patients in the hospital for other illnesses, a group of 26 medical students, a few of whom subsequently came down with influenza, and 9 patients with pulmonary tuberculosis. The medium used was for the most part heated blood agar (so-called "chocolate agar") made from a beef infusion basis, 5% defibrinated sheep blood and Witte's peptone (purchased before the war) and adjusted to a P_H of 7.4-7.6. In some instances unheated blood agar and Avery's oleate medium of the same H-ion concentration were also used. In addition to the plates from the nasopharyngeal swabbings, blood cultures and sputum examinations were made from some of the patients. In all instances the plates were streaked at the bedside, and the sputum examinations and animal inoculations were made without any material loss of time. An ordinary bent wire applicator was employed to obtain the material from the nasopharynx, and with reasonable care no contaminations were obtained by striking the swab against the tongue or other parts of the mouth.

A marked difference in the nasopharyngeal flora of the influenza patients was noted as compared with the results obtained by the same observers under essentially the same conditions during the more severe epidemic of 1918-19. The commonest organism in the throats of practically all the patients during the 1920 outbreak was a gram-negative coccus usually occurring in pairs. It was not infrequently present in almost pure culture on the chocolate-agar plates seeded with the nasopharyngeal swabbings. Organisms resembling B. influenzae were found only in small numbers in 10 instances. Members of the streptococcus-pneumococcus group were noticeable for their absence. They were rarely present, and only in relatively small numbers. In our

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1918-19 epidemic, on the contrary, hemolytic streptococci and virulent pneumococci prevailed largely in the throats of the influenza patients, as well as among the students not suffering from this malady.

This gram-negative coccus isolated from the throats of influenza patients resembled Micrococcus catarrhalis in its morphology and cultural characters. On chocolate agar it grew readily in the form of slightly elevated, moist, translucent, nonpigmented colonies. On ordinary beef extract agar and on Löffler's blood serum the organisms grew less rapidly than on the richer mediums, but an abundant growth was obtained in from 36 to 48 hours. No pigment was produced even on the Löffler's blood serum. Growth in broth was slight, and the organism failed to ferment any of the ordinary carbohydrates, as determined by reaction to litmus solution.

When injected intravenously into rabbits in amounts of 1 to 2 slant cultures per animal, the organisms produced a profound toxemia. The animals were completely prostrated within 12 hours after the injection. They would lie on their sides showing marked depression, rapid shallow respiration, usually with some diarrhea. Death occurred in from 2 to 3 days. No lesions, except cloudy swelling of the viscera and occasionally subserous hemorrhages in the intestines, were observed. Strikingly enough, in no instance, in spite of diligent effort, were the organisms recovered from the heart blood or other organs of the body.

The same organism from a morphologic and cultural basis was also found abundantly in the noninfluenzal patients in the hospital. Unfortunately, the virulence of these organisms was determined in only one case but in that instance, one 24-hour culture injected intravenously into a rabbit produced no untoward symptoms.

The cultures from the tuberculosis patients were all taken on Feb. 11, after the epidemic at the university had subsided. The sanatorium in which these patients were is several miles out of the city, and influenza was not present in this institution nor had any of the patients been in contact with the outside world except through the nurses, physicians and occasional visitors. The cultures from these cases looked about normal for the time of year as to the incidence of streptococci and pneumococci, but there were more gram-negative cocci than one usually finds. These organisms were for the most part the pigmented species, but in two cases we found numbers of organisms that morphologically, culturally and on the basis of carbohydrate reac-

tions were of the catarrhalis type. In one other instance we found an organism which grew like M. catarrhalis but fermented glucose, the carbohydrate reaction of the gonococcus. None of these organisms produced any symptoms more serious than a slight rise in temperature following inoculation of several cultures into rabbits.

Agglutination tests with the gram-negative cocci isolated from influenza patients and also a strain of Pfeiffer's bacillus were made with serum from six of the convalescents, but in no instance were positive results obtained.

A study of the nasopharyngeal flora of a group of 26 medical students was carried on weekly throughout the winter and early spring months with another purpose in view. These examinations were made by M. S. Allen and D. G. Conover with the idea of obtaining information as to a possible relation of the nasopharyngeal flora with meterologic conditions. Their findings need confirmation. It gives, however, additional information as to the incidence of M. catarrhalis or a similar organism among the general student population at this time. Ordinary blood-agar plates were used in this work, and a striking increase in the nonpigmented gram-negative cocci was observed during the epidemic period.

The epidemic began shortly after the return of the students from the Christmas vacation on Jan. 5, reached its peak with a total of 121 new cases Jan. 20, maintained approximately this level for 4 days, and then fell off, gradually disappearing altogether about Feb. 12 (Dr. J. S. Evans, personal communication). Large numbers of the catarrhalis type of organism were noticeable, especially during the peak of the wave, together with a marked falling off in the incidence of the members of the streptococcus-pneumococcus group. In fact, only 3 of the 26 showed any streptococcus-pneumococcus organisms at this time, whereas the average during the winter months showed a positive finding of these organisms in 12 of the 26. None of the organisms from this group were tested for virulence.

Obviously the results here reported suggest no etiologic relationship between the gram-negative coccus isolated and epidemic influenza. During this 1920 epidemic, however, this organism, which corresponds to the usual description of M. catarrhalis, was markedly enhanced in virulence by the growth under the conditions prevailing in the body of influenza patients. Similar organisms isolated from noninfluenzal cases at about the same time did not show this increased virulence. Whereas

ordinarily many cultures of this organism may be injected into rabbits without causing more than a slight transitory rise in temperature, in this instance doses as small as a single culture produced a fatal termination in 2 or 3 days. Apparently this is another rather striking instance of the capacity of the influenzal infection to increase the virulence of other associated organisms. This epidemic, as has been suggested by others, was less serious than the epidemic of a year before, presumably because of the low incidence of organisms that have the capacity of acquiring a high virulence for human beings, namely, streptococci and pneumococci such as were so abundantly present during the winter of 1918-19.

In view of these findings, it would seem as though gram-negative cocci other than the meningococcus and the gonococcus deserve more attention than they receive. Some years we have not infrequently found gram-negative cocci as the predominating organisms in throat cultures or in conjunctivitis or middle ear infections. Then for several years they may occur only rarely, and those present are usually the pigmented species. Should not all throat cultures, including those from cases of suspected diphtheria, be stained by Gram's method as a routine procedure? We believe that important additional information as to the rôle of M. catarrhalis and similar organisms would thus be obtained.